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Angiogenesis Inhibitors

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Angiogenesis inhibitors target the neovascular development that is hypothesized to underlie tumor growth. The inhibitors that are undergoing the clinical testing phase can be divided into five categories based on their target activity: 1) drugs that block matrix breakdown; 2) drugs that inhibit endothelial cells directly; 3) drugs that block angiogenesis activators; 4) drugs that inhibit endothelial cell integrins or survival signaling; and 5) drugs with a currently unknown mechanism of action. The properties of these drugs and some specific agents in each class are reviewed in this article. Because growth inhibition rather than tumor shrinkage is expected to be the clinical effect of angiogenesis inhibitors, some of the challenges and potential solutions for clinical trial design are also discussed.

Introduction

Angiogenesis inhibitors have been one of the largest sources of media fervor in the field of cancer research since US President Richard M. Nixon first declared the War on Cancer in 1971. In the same year, in fact, Judah Folkman and his colleagues demonstrated that tumor growth beyond 1mm in diameter requires development and growth of new blood vessels, a process termed neovascularization. Thus, the idea that one might potentially affect the ability of a cancer to grow by restricting its blood supply is not new, but the discovery of the natural angiogenesis inhibitors angiostatin and endostatin in 1997 elevated this concept to one of the hottest lines of cancer research in recent years. As a result, a number of angiogenesis inhibitors are in clinical development. Based on their putative mechanism of action, angiogenesis inhibitors can be classified into five different categories; 1) drugs that block matrix breakdown; 2) endothelial cell inhibitors; 3) drugs that block angiogenic activators; 4) drugs that inhibit endothelial-specific survival signaling; and 5) drugs with an unknown mechanism of activity. Updated information on the available clinical trials within each drug class is maintained on the Internet at the website of the National Cancer Institute of the US National Institutes of Health [1] (Table 1). In this review we discuss the rationale for each class and briefly describe those agents that appear most promising or are furthest along in their development. We end with a brief discussion of some of the challenges and possible solutions for clinical trial designs with these drugs, which are expected to lead to tumor growth inhibition, but not tumor shrinkage.

Drugs That Block Matrix Breakdown

The extracellular matrix (ECM) is a complex environment composed of proteins, including fibrinogen, collagen, and gelatin, that provide the supportive scaffolding of a tissue. The ECM undergoes frequent remodeling during normal and pathologic states including wound healing, response to inflammation, menstruation, and tumor growth. ECM degradation is accomplished by a group of proteins termed matrix metalloproteinases (MMPs). MMPs constitute a family of at least 26 enzymes that are categorized as matrilysins, stromelysins, and gelatinases. Active at a neutral pH and dependent on calcium and zinc for catalytic activity, MMPs exist as either secreted or transmembrane proteins. The former are secreted in a latent proenzyme form, and cleavage is required for the enzymes to become active in vivo. MMPs can likewise be bound and inactivated by a series of natural inhibitors, called tissue inhibitors of metalloproteinases, or TIMPs. With respect to tumor neovascularization, it would appear logical that growth of new vessels into a tumor requires remodeling of the surrounding ECM. Not surprisingly, then, a large number of reports have documented increased expression of both MMPs and TIMPS in tumor tissue [2–6]. Although increased expression of TIMPs may be counterintuitive, it probably represents a compensatory response to the elevated expression of MMPs. In fact, results from the few studies that examined this issue indicate that the ratio of MMP to TIMP activity appears to be higher in tumor than in the corresponding normal tissue [5,6]. Based on these findings, a number of MMP inhibitors have been investigated for their effect on tumor growth and metastasis.

The related compounds marimastat and batimastat (British Biotech, Oxford, UK) have been most extensively investigated. They inhibit MMPs by chelating zinc and preventing it from binding to the catalytic site. In animal models, these compounds inhibit tumor invasion, angio-

Table 1. Angiogenesis Inhibitors

Angiogenesis inhibitor*	Manufacturer/ sponsor/location	Clinical trial status	Mechanism
Batimastat	British Biotech, Oxford, UK	Phase I	MMP inhibitor; chelates zinc and prevents activation of proenzymes
Marimastat	British Biotech	Phase III	MMP inhibitor; chelates zinc and prevents activation of proenzymes
Bay 12-9566	Bayer, West Haven, CT	Phase II/III	MMP-2 and MMP-9 inhibitor
AG3340	Agouron, La Jolla, CA	Phase III	MMP-2,3,9,13,14 inhibitor
TNP-470	TAP Pharmaceuticals, Deerfield, IL	Phase I Phase II	Inhibits endothelial cell growth
Thalidomide	NCI	Phase II	Unknown
Squalamine	Magainin Pharmaceuticals, Plymouth Meeting, PA	Phase I Phase II	Inhibits sodium-hydrogen exchanger, NHE3
Endostatin	EntreMed, Rockville, MD	Phase I	Inhibits endothelial cell growth
Combretastatin A-4 (CA4P)	OXiGENE, Cambridge, MA	Phase II	Inhibits tubulin polymerization
SU5416	Sugen, South San Francisco, CA	Phase I/II	Specific inhibitor of the Flk-1/KDR VEGF receptor
SU6668	Sugen	Phase I	Specific inhibitor of the Flk-1/KDR VEGF receptor
PTK787/ZK 22584	Novartis, London, UK	Phase I Phase I/II	Specific inhibitor of the Flk-1/KDR VEGF receptor
Interferon-α	NCI	Phase II/III	Blocks VEGF and bFGF expression
Anti-VEGF antibody	NCI	Phase II	A monoclonal antibody against the αvβ3 integrin
Vitaxin	Ixsys, San Diego, CA	Phase II	A monoclonal antibody against the αvβ3 integrin
EMD121974	Merck, Whitehouse Station, NJ	Phase I/II	Prevents ligation of the αvβ3 integrin
CAI	NCI	Phase I Phase II	Synthetic inhibitor of nonexcitable calcium channels
Interleukin-12	Genetics Institute, Cambridge, MA	Phase I/II	Promotes induction of a TH1 immune response

*Phase of testing is noted for each agent, but multiple trials may be ongoing in different types of cancer. bFGF—basic fibroblast growth factor; MMP—matrix metalloproteinase; NCI—National Cancer Institute of the US National Institutes of Health; VEGF—vascular endothelial growth factor. *Adapted from* NCI [1].

genesis, and growth [7]. Because of its better oral bioavailability, marimastat was selected for human trials. Phase I studies showed that major toxicities were musculoskeletal pain and inflammatory arthritis [8,9]. Phase II studies in patients with advanced colorectal, prostate, pancreatic, and ovarian cancer demonstrated no significant objective responses, but an effect on the rate of rise of serum tumor markers was suggested [10]. Although some doubts remain as to the validity of these observations, large-scale phase III studies of standard chemotherapy with or without marimastat are currently underway. AG3340 (Agouron Pharmaceuticals, La Jolla, CA), a synthetic MMP inhibitor of MMPs 2, 3, 9, 13, and 14 based on MMP x-ray crystal structure, has potent anti-angiogenic effects in animal models and has undergone phase I testing [11,12]. Once again, the dose-limiting toxicity shown in this clinical trial was joint pain, which thus seems to be a common effect of MMP inhibition. Bay 12-9566 (Bayer, West Haven, CT) is an inhibitor of MMP 2 and 9 that also has activity in animal models. Toxicities found in phase I trials include thrombocytopenia and transaminitis [13]. Phase II and III studies have been initiated simultaneously. Compounds in earlier stages of clinical development are listed in Table 1.

Drugs That Inhibit Endothelial Cells Directly

Because of their unique biologic properties, tumor-associated endothelial cells are another attractive target for drug development. The most prominent endothelial cell inhibitors are endostatin and angiostatin. The discovery of endostatin followed closely on the heels of the isolation of angiostatin by the same group of researchers. Endostatin is a fragment of collagen XVIII and is a potent inhibitor of endothelial cell growth [14•]. Initial studies of endostatin also show that repeated treatment cycles of tumor-bearing animals with endostatin did not select for a resistant tumor population [15••]. These results raised the hypothesis that prolonged therapy of genetically stable endothelial cells may be more efficacious than prolonged therapy of genetically unstable tumor cells. Phase I studies of endostatin in cancer patients are underway.

Thalidomide, despite its rocky history, has recently made a comeback as a treatment for leprosy and AIDS-related apthous ulcers. Thalidomide has a myriad of immunologic and cellular effects, including inhibition of tumor necrosis factor production [16]. In 1994, antiangiogenic activity was reported with thalidomide in a rabbit cornea micropocket assay [17]. Although other investigators have not confirmed its antiangiogenic properties, clinical studies in cancer patients have proceeded nonetheless. Results from phase II studies suggest that thalidomide may be effective in the treatment of Kaposi's sarcoma, glioblastomas, and multiple myeloma [18–20]. It is unclear whether this activity is caused by an antiangiogenic mechanism. Its major toxicity is somnolence.

Another potent endothelial cell toxin, fumagillin, was first discovered as the product of a fungal contaminant in endothelial cell cultures [21]. It covalently binds a metalloprotease, methionine aminopeptidase, but the relationship of this observation to the antiangiogenic properties of fumagillin is unknown. Since its discovery, several synthetic analogues of fumagillin have been developed; among those, TNP-470 was selected for further development. TNP-470 has shown potent antitumor effects in animal models and has been evaluated in patients with Kaposi's sarcoma, cervical carcinoma, and renal cell carcinoma [22–24]. Its toxicity profile includes asthenia, fatigue, vertigo, and loss of concentration. Although overall response rates were low in the clinical studies, the suggestion of prolonged stable disease as a specific drug effect was made.

The final two endothelial cell toxins in clinical trials are combrestatin A-4 and squalamine. Squalamine, a dogfish shark extract derivative, is a broad-spectrum water-soluble antibiotic that inhibits the sodium-hydrogen exchanger, NHE3 [25,26]. Phase I studies showed that its toxicities include fatigue, low-grade nausea, and anorexia [27]. Squalamine is currently being tested in phase II clinical trials for efficacy against non–small-cell lung cancer. Combretastatin A-4 is a natural product derivative from the African tree, *Combretum caffrum*, and is a powerful inhibitor of tubulin polymerization. It can cause tumor-specific vascular damage by targeting proliferating endothelial cells [28,29]. Combrestatin A-4 is currently undergoing phase I clinical trials.

Drugs That Block Activators of Angiogenesis

A number of molecules have been identified that induce angiogenesis. In normal tissue, during wound healing or menstruation, these molecules are produced, and stimulate endothelial cell proliferation. Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF) are the most important of these soluble growth factors, and their expression is often upregulated in tumor tissue. Development of drugs that specifically inhibit these factors or the binding to their receptors has thus been an active research endeavor.

SU5416 (SUGEN) is a potent and specific inhibitor of the Flk-1/KDR VEGF receptor. It inhibits endothelial cell growth but not growth of a variety of tumor cells in vitro [30]. Growth of a number of human tumor xenografts is also inhibited in vivo [30]. Phase I trials in humans suggest that the drug is well tolerated, with the major toxicity being headache and fatigue. Phase II trials in a number of malignancies are planned. PTK787/ZK 22584, another VEGF receptor blocker, is undergoing phase I trials against glioblastoma multiformae and Kaposi's sarcoma. A second antiangiogenesis drug, SU6668, has multiple growth factor receptor targets, including the Flk-1/KDR, platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) receptors. SU6668 is currently undergoing phase I clinical studies.

A second strategy for blocking angiogenic molecules is through the use of naturally occurring inhibitors. Interferon-α (IFN-α) inhibits endothelial cell migration and proliferation by blocking VEGF and bFGF expression [31••]. Activity of IFN-α in renal cell carcinoma, melanoma, and multiple myeloma is well recognized. Whether this activity is caused by its antiangiogenic effects or its immunologic effects remains unclear. A monoclonal antibody to VEGF has also been developed as an antiangiogenic agent. Treatment of mice carrying a human prostate cancer xenograft with the antibody demonstrated complete inhibition of tumor growth after the preangiogenic growth phase [32]. The antibody is currently in phase II clinical trials against renal cell carcinoma.

Drugs That Inhibit Endothelial-specific Integrin/Survival Signaling

Rather than preventing endothelial cells from proliferating and migrating, a different strategy that targets these same cells addresses their ability to survive. Endothelial cells have a complex relationship with the extracellular matrix that requires very specific scaffolding and signaling elements not only for growth but also for survival. In particular, the integrins, a large family of transmembrane glycoproteins, are critical mediators of cell-ECM interactions. Specifically, the αvβ3 integrin is required for the survival and maturation of new blood vessels [33,34••]. Two drugs have been developed that block the integrins expressed at the surface of endothelial cell surfaces, preventing endothelial cells from interacting with the ECM.

Vitaxin is a humanized monoclonal antibody against the αvβ3 integrin. Preclinical studies demonstrated its ability to reduce TGFβ expression, enhance apoptosis, and cause shrinkage in vessels in a rabbit model following balloon angioplasty (injury of the iliac arteries) [35]. Phase I studies in 15 patients with solid tumors showed that the drug was well tolerated; infusion-associated fever was the only side effect [36]. Phase II trials for this agent are now being planned.

Another antagonist to $\alpha v\beta 3$ is EMD121974, a small cyclic peptide that prevents ligation of the $\alpha v\beta 3$ integrin, which in turn prevents maturation and promotes apoptosis of proliferating endothelial cells [33]. In preclinical studies, EMD121974 abolished the expression of $\alpha v\beta 3$ on the cell surface and had a synergistic effect when used in conjunction with tumor-specific interleukin-2 immunotherapy in a murine neuroblastoma model. This agent is currently in phase I/II trials against Kaposi's sarcoma, brain tumors, and solid tumors.

Drugs with Nonspecific Mechanism of Action

In addition, several antiangiogenic compounds are in development for which the mechanism of angiogenesis inhibition is unknown. Carboxyamido-triazole (CAI), formerly developed by Merck as L651582, is a synthetic inhibitor of nonexcitable calcium channels. CAI has been shown to reversibly inhibit angiogenesis in a human umbilical vein endothelial cell (HUVEC) model, as well as the chick chorioallantoic membrane (CAM) assay, two standard tests of antiangiogenic ability [37]. Studies in prostate, glioblastoma, and breast cancer cell lines have shown that CAI inhibits proliferation of these lines as well, and thus its effects may not be unique to endothelial cells [38–40]. Phase I studies of CAI show dose-limiting neurocerebellar toxicity (ataxia), as well as fatigue, nausea, and vomiting [41,42].

Interleukin-12 (IL-12) has also been explored as an antiangiogenic compound. IL-12 is a disulfide-linked heterodimeric pleiotropic cytokine produced by B-cell lymphocytes and macrophages. It binds to a receptor on T-cells and natural killer (NK) cells, promoting the induction of a TH1 immune response [43]. IL-12 has been shown to suppress tumorigenicity in several animal models, and it may exert its antitumor activity by priming macrophages to produce nitric oxide (NO) [43,44]. Although the primary method of IL-12 antitumor activity is probably through induction of an immune response, there is also evidence that IL-12 may be working through a separate antiangiogenic pathway to inhibit tumor growth [45,46]. In patients, IL-12 administration has been complicated by tachyphylaxis, in which tolerance to its toxic effects develops rapidly after the initial dose [47]. Further clinical development of IL-12 is ongoing.

Clinical Trial Design and Angiogenesis Inhibitor Studies

The vast majority of preclinical studies with antiangiogenic agents demonstrate that these agents inhibit tumor growth but only rarely cause any tumor shrinkage. Thus, the most likely clinical effect of these agents will be disease stabilization. This phenomenon, however, raises significant issues with respect to study design, especially for phase II trials. Such trials use surrogate end points to determine whether an agent has sufficient activity to warrant more definitive phase III trials, in which patient benefit can be assessed. Because phase III trials require end points of survival, time to progression, or quality of life, enrollment of a large number of patients is necessary. This is a time-consuming and expensive endeavor, and one must choose wisely which agents to advance from phase II to phase III testing. Such choices can only be made with optimal phase II data. The most common phase II surrogate end point for traditional cytotoxic antineoplastic agents has been radiographic tumor shrinkage, with an arbitrary 50% decline in the sum of orthogonal measurements defining "objective response." Because the expected response rate with this measure (*ie*, the null hypothesis) in the absence of treatment is 0, design of a trial that can statistically reject the null hypothesis and conclude that the agent is worthy of further study is relatively straightforward. For a cytostatic antiangiogenic agent, the simplest design would be to define response as any patient who does not experience disease progression. The major problem with this approach is that it is very difficult to determine what the appropriate null hypothesis should be. Most malignant diseases have a highly variable clinical history, with a certain percentage of patients who will maintain stable disease even in the absence of treatment. Such patients usually cannot be identified on study entry and tend to be overrepresented in clinical trials. This phenomenon was illustrated in our study of TNP-470 in metastatic renal cancer: six of 33 patients maintained long-term stable disease, but we were unable to conclude whether this was a specific drug effect [48]. We, as well as other investigators, have considered these issues in detail [49•]. Although an extensive discussion is beyond the scope of this review, we argue that phase II testing of antiangiogenic agents should proceed in two steps. In the first step (phase IIA trials), determination should be made of whether cellular target of the agent is inhibited in human patients. Ideally, one would like to demonstrate that tumor neovascularization is inhibited (in either tumor biopsy specimens or via novel radiologic imaging methods). In the second step (phase IIB trials), disease stabilization can be assessed, but this would require a randomized design. We have advocated a randomized discontinuation design for such trials in which all patients are treated with the agent of interest, and those who maintain stable disease for a defined period, *x*, will then be randomized either to continuing or discontinuing the agent. The primary end point is then the rate of stable disease in the two groups after an additional time, *x*. We have estimated that such a trial can be completed with approximately 60 to 100 randomized patients. Although other randomized phase IIA designs are possible, they are all larger and more expensive than traditional phase IIB trials. Thus, significant resistance exists for such an

approach. However, this design is a far more efficient and less costly approach then initiating phase III trials with inadequate data.

Conclusions

Angiogenesis inhibitors constitute a promising line of anticancer research. A broad spectrum of synthetic and natural inhibitors has been identified, and preclinical studies have brought encouraging results. Perhaps the most exciting aspect of these studies is the potentially slower development of resistance when an inherently genetically stable cell population is targeted. A number of these inhibitors are already undergoing clinical trials, which have demonstrated their tolerance. Further development will probably require approaches that are significantly different from the strategies involved in development of traditional cytotoxic agents.

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